Effects of Artificial Feeding and Copper Sulfate on Nutrients and Dissolved Oxygen in Fish Ponds

Research Thesis

Presented in partial fulfillment of the requirements for graduation *with research distinction* in Biology in the undergraduate College of Arts and Sciences of The Ohio State University

by

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June 2011

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Abstract

Artificial feed is commonly added to aquaculture ponds to enhance growth and survival of juvenile fish. However excessive feeding may decrease fish growth and survival by reducing water quality and natural prey populations. Feeding also introduces excessive nitrogen and phosphorus into ponds, which promote growth of nuisance phytoplankton and cause reduced oxygen conditions from aerobic decomposition of feed. Copper sulfate, which is often used to control phytoplankton growth, may also contribute to low dissolved oxygen by reducing algal primary production.

In this study, we determined oxygen consumption from feed decomposition in a closed system, and the effects of artificial feed on spatial concentrations of dissolved oxygen (DO) in the ponds. We quantified the nitrogen and phosphorus release from artificial feed over time, and the effects of copper sulfate application on distribution and consumption of DO. To calculate DO consumption, we incubated known quantities of feed with pond water in biological oxygen demand (BOD) bottles for 6 hours, followed by measuring DO in each bottle using Winkler titrations. We assessed the effects of artificial feeding on the morning DO variability (8:00 am) in ponds receiving different feeding rates (0%, 1%, or 3% of body weight per day). To estimate nitrogen and phosphorus release from feed we suspended samples in mesh bags within the pond for 0, 1, 2, 4, 8, 16, and 32 hours and measured changes in remaining nitrogen and phosphorus content. We measured oxygen consumption and distribution before and after copper sulfate addition.

Oxygen consumption increased rapidly with feed additions in BOD bottles suggesting high feeding rates could have a negative effect on DO in ponds. Spatial variation in DO was likely caused by artificial feeding, with low DO localized near the site of feed addition. Fish may have had limited access to feed in hypoxic areas and were observed gasping for air at the surface. Phosphorus and nitrogen were released at a molar ratio of 10:1, which potentially supported the growth of filamentous algae (*Rhizoclonium sp*.) observed in ponds. Copper sulfate application likely reduced DO because it can reduce algal primary production in ponds. Managers should consider reducing the rate of artificial feeding, and avoiding treatment with copper sulfate in order to improve water quality and growth and survival of fish. Further research is needed to determine optimal feeding rates that balance fish growth with water quality.

Introduction

Aquaculture is growing rapidly on a global scale (FAO 2011). Freshwater fish culture in the United States, used for sport fish stocking and commercial production (USDA/NASS 2007), mainly occurs in small ponds that often receive fertilizer and/or artificial feed additions to increase fish yield beyond what is provided by *in situ* prey production (Diana et al. 1991, and Swingle and Smith 1939). Ideally, artificial feeding would be managed to balance fish production with maintenance of suitable habitat quality. However, not all nutrients from artificial feed are assimilated into fish tissue and are instead released into the pond environment (Cole and Boyd 1986). For example, Boyd (1985) found that fish assimilated only 26.8% nitrogen and 30.1% phosphorus from feed in catfish culture ponds. Nitrogen and phosphorus released from feed can thus stimulate algal growth beyond desirable levels (Culver 1991, Culver 1996, and Levich 1996), at which point managers often add algaecides such as copper sulfate to control algal biomass (Flemming and Trevors 1989). Furthermore, high feeding rates can cause hypoxia (dissolved oxygen <2 mg L^{-1}) in ponds, through aerobic decomposition of unconsumed or unassimilated feed or by encouraging dense growth of Cyanobacteria (Paerl and Tucker 1995). While artificial feeding is an integral component of freshwater fish culture, and is usually needed to sustain profitable yields (Swingle et al. 1954), its negative effects on water quality and nitrogen and phosphorus geochemistry are rarely considered when managing ponds. As such, a better understanding of these effects is needed to develop optimal feeding strategies.

In this study, we quantified the rates of oxygen consumption from bacterial decomposition of feed in closed systems, as a function of feeding rate. We determined the effects of artificial feeding on spatial distribution of DO in fish ponds. We identified the relative and total amounts of nitrogen and phosphorus released from artificial feed in ponds, and we identified the effects of copper sulfate on DO consumption and distribution.

Objective 1: Effects of artificial feed on oxygen consumption and spatial distribution of DO

The morning DO concentration in commercial catfish ponds decreases with increasing rate of application of artificial feed (Boyd 1982, and Cole and Boyd 1986). While hypoxia is known to directly reduce the growth rate and survival of fish (Andrews et al. 1973, Boyd et al. 1978) the indirect effects of nutrient release from unassimilated artificial feed on water quality and lower trophic level dynamics are rarely considered in intensive aquaculture ponds. Nutrients released from feed can stimulate aerobic bacterial respiration, thus locally consuming DO (Boyd 1985). Organic fertilizers such as alfalfa meal, which are commonly applied to ponds to increase primary and secondary productivity, often result in hypoxia through breakdown of organic material (Qin and Culver 1994). Managers often use aeration to combat low DO, but heavy aeration can have negative effects on water quality through re-suspension of sediment and erosion of pond bottoms (Boyd 1998). Although correlations exist between the rate of artificial feeding and pond water quality, the spatial distribution of DO in ponds has not been adequately described. In this study, we determined the effects of artificial feeding on the rate of DO consumption, and the spatial concentrations of DO in fish ponds. Understanding these concepts will likely improve reliability and efficiency of fish production.

Objective 2: The effects of artificial feed on nitrogen and phosphorus dynamics in fish ponds

Artificial feeding releases phosphorus and nitrogen into fish ponds which may modify lower trophic-level dynamics. Phosphorus has been shown to limit primary productivity in freshwater ecosystems (Schindler 1971), such that strong correlations exist between total phosphorus concentration and chlorophyll a, which is a proxy for algal biomass (Schindler 1978). In lakes with excessive phosphorus levels, nitrogen may limit primary productivity (Smith 1984), such that nitrogen addition increases phytoplankton growth (Lin 2008). In catfish ponds, nitrogen and phosphorus inputs from feeding may cause excessive algae or vascular plant growth that interferes with routine pond management activities (e.g., feeding and harvesting fish). Also, high primary productivity or sudden phytoplankton bloom die-offs can cause periodic hypoxia and high levels of toxic free ammonia that decreases fish growth and survival (Boyd et al. 1975).

The ratio of nitrogen to phosphorus (N:P) can strongly influence the competitive advantage for growth of certain species of phytoplankton over others (Culver 1991, and Smith 1993). Many Cyanobacteria that produce toxins that can cause fish kills (Culver 1996, and Zimba 2001) or odorous metabolites responsible for off-flavors in fish tissue (Lovell and Sackey

1973), typically thrive at low N:P ratios or extremely high phosphorus levels (Smith 1983). Filamentous algae (e.g., *Rhizoclonium sp.*) often experience excessive growth in low N:P conditions and can interfere with management activities particularly during harvest by forming dense floating mats on the surface of ponds (Culver 1996). Zooplankton are important prey for larval and juvenile fish, therefore maintenance of N:P ratios that promote growth of edible phytoplankton (prey items for zooplankton) is necessary for proper management of lower trophic level dynamics to promote good growth of higher consumers (cultured fish). For these reasons, understanding the relative and absolute rates of nitrogen and phosphorus release from artificial feed into the pond environment is critical to assessing effects on water quality and fish production in ponds. In this study, we determined the rate and quantity (absolute and relative) of nitrogen and phosphorus release from artificial feed to predict how excessive feeding may influence nitrogen and phosphorus biogeochemistry and phytoplankton community composition.

Objective 3: Effects of copper sulfate application on the consumption and distribution of DO

Copper sulfate ($CuSO_4$) is often added to fish ponds to control excessive growth of algal primary producers, effectively reducing phytoplankton biomass (Flemming and Trevors 1989), and is also commonly used to treat the protozoan parasite *Ichthyophthirius multifiliis* in catfish culture ponds (Schlenk et al. 1998). Infection with *I. multifiliis* reduces growth and survival of catfish, due to the high energetic cost (parasite load) of fighting off the infection. An *in situ* $CuSO_4$ concentration of 0.4 mg L^{-1} is sufficient to treat this infection, resulting in the mortality of *I. multifiliis* (Schlenk et al. 1998). Managers also use $CuSO_4$ to prevent “off-flavors” in catfish flesh caused by cyanobacterial metabolites. $CuSO_4$ is the only chemical treatment that reliably reduces biomass of primary producers and effectively eliminates off flavors in cultured catfish

(Tucker and Hergreaves 2003), and is the only algaecide approved for use in catfish ponds by the US Environmental Protection Agency (U. S. EPA 2003). Organisms other than phytoplankton are also sensitive to $CuSO_4$. Primary consumers such as zooplankton, and higher level trophic organisms, such as rainbow trout and channel catfish, vary in their sensitivity to copper concentrations (Smith and Heath 1979). However, because copper is also a micronutrient, its toxicity is buffered by metabolic processes in vertebrates.

Treatment with $CuSO_4$ may deteriorate water quality by reducing primary production, thereby causing oxygen deficiencies in ponds, and may have undesirable long term effects with continued applications (Tucker and Boyd 1978). Accumulation of $CuSO_4$ occurs in the sediment with continued applications, which can cause long-term reduced bacterial activity and a broad spectrum of other adverse effects (Han et al. 2001). Toxicity of $CuSO_4$ to phytoplankton often results in the death of much of the population of phytoplankton. Dead phytoplankton stimulate bacterial respiration and thus further reduce DO in fish ponds often leading to decreased growth and survival of fish (Schrader et al. 2003). Managers must understand the effects of $CuSO_4$ on oxygen dynamics in fish ponds to effectively make management decisions about its proper application.

Predictions

Objective 1: DO consumption in BOD bottles will have a positive correlation with feeding rate. DO concentrations will be lower in high feeding rate ponds. DO will be spatially heterogeneous, and DO deprived zones will exist where the feed applied to the pond.

Objective 2: Nitrogen and phosphorus will be released from feed at high rates initially and reduced rates over time. The ratio of N:P release from feed will be low.

Objective 3: Oxygen consumption in BOD bottles and DO concentrations in the ponds will be lower after $CuSO_4$ application to ponds due to reduced algal biomass.

Methods

Study site:

Our pond experiments were conducted at Hebron State Fish Hatchery, Ohio, which is managed by the Ohio Division of Wildlife (DOW). Ponds were filled one week prior to fish stocking, using water routed from a local eutrophic reservoir, Buckeye Lake, via an extant section of the Ohio Erie Canal. High nutrient levels and low DO concentration in the source water, which frequently approaches 0 mg/L in summer months (Pat Howard, DOW, personal communication), cause subsequent problems with water quality in ponds that have resulted in historically variable fish production at this hatchery. The molar ratio of nitrogen to phosphorus (N:P) in the source water is often less than 22:1 during the fish production season (Filbrun et al. 2008).

Age-0 channel catfish, *Ictalurus punctatus*, were fed 3%, 1%, and 0% body weight per day in separate experimental ponds. These feeding rates were adjusted weekly to match fish growth, and were calculated assuming no mortality, meaning highest feeding rates occurred just before harvest. The artificial feed that was used for these experiments, Silver Cup Fish Feed (Nelson & Sons, Inc., Murray, UT), is widely used for a variety of intensively cultured fish species. These experiments were conducted during September because maximum feeding rates (6.8, 2.7, and 0 kg feed day^{-1} for the 3%, 1%, and 0% body weight day^{-1} ponds respectively) occurred at this time. The field experiments began 31, August 2010, and ended on14, September 2010.

Objective 1: Effects of artificial feed on oxygen consumption and spatial distribution of DO

In order to estimate the amount of oxygen consumed by bacterial digestion of the artificial feed, biological oxygen demand (BOD) bottles were incubated with pond water and artificial feed. We considered that feed does not diffuse to the entire pond volume, but rather to local volumes near the site of feed application. Based on the common feeding rate of 3% body weight per day (1.5 mg L^{-1} calculated from known feed additions and total pond volume) we calculated the amount of feed per unit volume using average kettle (a deep concrete trough used to capture fish during pond draining and also the site of feed addition) volume as a standard. We determined the amount of feed to be added to each bottle to model diffusion of nutrients from feed across multiples of a kettle volume, ranging from 0.5 kettles to 10 kettles. Experimental BOD bottles filled with pond water and feed additions of 0.015, 0.03, 0.15, and 0.3 mg feed L^{-1} were incubated *in situ* in the 1% feeding rate pond for 6 hours alongside control bottles with no feed addition. Respiration due to artificial feed addition (Figure 1) was calculated by comparing respiration between the experimental and control bottles. DO was measured in all bottles using the Winkler DO test without azide modification (APHA 2005).

The distribution of DO concentrations (Figure 2) were monitored in three age-0 catfish culture ponds (a 3%, 1%, and 0% feeding rate pond) in the morning and afternoon at multiple locations on a single date. Ponds were not aerated on the sampling date, but were aerated on other dates when DO approached 0 mg L^{-1} near the kettle. Dissolved oxygen was measured using an air calibrated electrode sonde attached to a YSI Model 556, at the surface and 1-m depth at the intersections of 5-m gridlines within a subset (30m x 30m x 1m volume) of the ponds (Figure 2) that included the site of feed addition. Spatial variation in DO (Figure 3) was shown using interpolation between measurement points, performed by SigmaPlot (Systat Software, Inc., Chicago, Illinois). The median of DO concentrations was calculated using these data points (Table 1). Temperature was also recorded with each DO measurement.

Objective 2: The effects of artificial feed on nitrogen and phosphorus dynamics in fish ponds

Amounts of total nitrogen and total phosphorus released from feed were calculated by comparing nutrient mass in samples of feed incubated in ponds for known times relative to the nutrient mass in the initial dry feed. Known quantities of artificial feed (Silver Cup No. 3) were wrapped in bags made of 64-μm nylon mesh and were suspended within the ponds. Based on evidence from similar nutrient-release experiments using alfalfa meal (Qin and Culver 1994), we anticipated that nutrient release would occur rapidly during the first day of incubation. Furthermore, Qin and Culver (1994) showed that over time organic fertilizer can accumulate bulk nitrogen mass from other sources, likely as feed becomes colonized with nitrogen-assimilating or even nitrogen-fixing bacteria. This study avoided such accumulations by using short incubation periods of only 32 hours compared to several weeks in other studies. We collected bags of feed at 0, 1, 2, 4, 8, 16, 32 hours to characterize the rate of nutrient release. Three replicates were performed for each feed incubation time. Bags were filled with 75 g artificial feed (Silver Cup No. 3) at time 0, and placed randomly in the 1% feeding rate pond at least 1m apart.

In the laboratory, feed remaining in each bag was removed and dried for 4 days at 60^{o}C or until constant weight. The change in feed mass was calculated by subtracting the final sample masses from the initial masses. Dried feed samples were then sent to Brookside Laboratories, Inc. (New Knoxville, Ohio) for total nitrogen and phosphorus determination. Total nitrogen was determined by combustion analysis, using Elementar vario MAX (Elementar, Hanan, Germany). For total phosphorus analysis feed was digested with nitric acid in a laboratory CEM microwave and analyzed in a Thermo ICP-MS (Fisher Scientific, Hampton, New Hampshire). Nitrogen and phosphorus release were calculated by subtraction of masses of total nitrogen and phosphorus in incubated feed (final), from the masses measured in non-incubated feed (initial).

Percent of feed released during the 32-h incubation was calculated by dividing the masses of nitrogen and phosphorus remaining in incubated feed by the initial masses measured in dry feed. The N:P molar ratio was calculated using the masses of remaining feed and concentrations of N and P (Figure 4).

Objective 3: Effects of $CuSO_4$ application on the consumption and distribution of DO

Ponds were treated with 0.33 mg L^{-1} $CuSO_4$ twice (3 September and 10 September). BOD bottles were filled with $CuSO_4$-treated water and with feed additions of 0.015, 0.03, 0.15, and 0.3 mg feed L^{-1}, then incubated *in situ* for 6 hours alongside control bottles with no feed addition (see methods for Objective 1). Spatial variation in DO in ponds with different feeding rates was measured before and after ponds had been treated with $CuSO_4$, to assess the effects of copper on DO dynamics.

Statistical analysis:

We tested for differences in oxygen consumption in BOD bottles with different feed treatments using one-way ANOVA with Tukey's HSD test (5% familywise type-I error rate) to detect pair-wise differences among treatments. Data were tested for the assumption of normality and equality of variances and transformed when necessary. Statistical analyses were performed using the statistical computer package SPSS Statistics Version 17.0 (SPSS Inc., Chicago, IL). We modeled nutrient release using a regression model with time factors.

Results

Objective 1: Effects of artificial feed on oxygen consumption and spatial distribution of DO

Oxygen consumption in BOD bottles increased with increasing amounts of feed added. Over a 6 hour period, BOD had a positive curvilinear relationship with the amount of feed added (Figure 1). In the high feed treatment, DO in bottles approached 0 mg L^{-1}, with a maximum oxygen consumption rate of 3.5 $mgL^{-1}6h^{-1}$. This suggests that oxygen may have limited oxygen consumption and bacterial respiration in bottles in high feed treatments.

Ponds were spatially heterogeneous in DO concentrations, which varied from highest values at the surface to lowest values at the bottom (Table 1). The 0% feeding rate pond exhibited the highest available oxygen in the morning and the afternoon. The 3% feeding rate treatment experienced significantly lower DO concentrations; values were especially low near the kettle in the morning (Figure 3). The 1% feeding rate pond exhibited intermediate DO concentrations between those of the 3% and 0% feeding rate ponds. Dissolved oxygen approached 0 mg L^{-1} at the bottom of ponds (1% and 3% feeding rate ponds) and approached saturation at the surface (0% feeding rate ponds). These values show that ponds are not uniform habitats, and that DO concentrations can vary significantly over space. Results also show that high feeding rate correlates with low DO in ponds. A heterogeneous pond environment and low DO can be problematic for cultured fish which require oxygen to grow and survive. Morning DO concentrations approached zero near the kettle where feed was applied to ponds, and DO increased with increasing distance from the kettle (Figure 3). Areas farthest from the kettle always contained the highest DO in all treatments whereas areas closer to the kettle always contained the lowest DO concentrations.

Objective 2: The effects of artificial feed on nitrogen and phosphorus dynamics in fish ponds

Unassimilated artificial feed released excessive nitrogen and phosphorus into pond environments. The initial N:P molar ratio in dry feed (Silver Cup no. 3) and release of nitrogen and phosphorus during the first 32 hours of incubation was constant at 10:1, which is low enough to favor growth of cyanobacteria in lakes (Smith 1983). This ratio remains constant through time, which suggests that nitrogen and phosphorus were released by heterotrophic assimilation rather than as inorganic forms. Release of nutrients from feed showed a curvilinear pattern (Figure 4). Nitrogen and phosphorus were released quickly during the first hour of incubation, and more slowly released thereafter.

Objective 3: Effects of $CuSO_4$ application on the consumption and distribution of DO

BOD increased with increasing amounts of feed in bottles with $CuSO_4$ treated pond water; however, mean BOD rates were comparatively greater before $CuSO_4$ addition for each level of artificial feed tested (independent samples t-test, two-sided $p < 0.05$). A positive linear relationship between artificial feed and BOD suggests that oxygen did not become limiting in copper-treated BOD bottles, possibly owing to the reduced biomass of bacteria (Figure 1). Reduction of BOD can be explained by reduced phytoplankton and bacterial respiration.

Dissolved oxygen concentrations in $CuSO_4$-treated ponds were lower than those before $CuSO_4$ application. The DO concentration in the entire monitored volumes in the 1%, and 3% feeding rate ponds were hypoxic (Figure 3), which is unsuitable for good growth and survival of catfish (Andrews et al. 1973). All three ponds showed a decrease in median of DO concentrations after $CuSO_4$ was added (Table 1). This decrease in DO is not likely due to changes in solubility of oxygen at different temperatures because temperature was lower in the $CuSO_4$ treated ponds, and colder pond water would have higher oxygen solubility.

Discussion

Objective 1: Effects of artificial feed on oxygen consumption and spatial distribution of DO

In the BOD bottle experiment, artificial feeding reduced DO through bacterial activity. By extrapolation, more feed would lead to lower DO in the entire pond (or a small subset of the pond in which the feed can be broken down). Pond systems, similarly to bottles, would be limited by the amount of available DO, which could potentially approach zero where feed is being broken down. This estimation is consistent with the results of the oxygen distribution experiment which showed that higher feeding treatments resulted in lower DO concentrations near the kettle.

Artificial feed is added to ponds to increase fish yield; hypoxic conditions, however, which result from high feeding rates may cause a reduction in fish growth and survival (Secor and Gunderson 1998). Consistent with other studies, our results showed increased feeding rates can decrease DO concentrations in fish culture ponds, which may cause decreased fish growth and survival (Boyd 1982). Hypoxic conditions may not provide a suitable environment for the survival of cultured fish. In our study, the entire monitored area of the 3% feeding rate pond was hypoxic (before and after $CuSO_4$ addition), and thus poses the risk of reduced of fish growth and survival.

Other studies have shown the negative correlations of feeding with DO concentrations (Boyd 1982, and Cole and Boyd 1986), but our study shows that the effect is not uniform across an entire pond. We found that oxygen depletion in pond water is localized to areas where feed is applied (kettle). Hypoxic conditions are more extreme near the kettle and decrease in severity with increased distance from the kettle. One implication of the gradient in DO concentrations is that fish may spend more time in areas further away from the kettle, effectively avoiding low DO and high stress conditions. However, fish may not have access to feeding sites due to restrictions imposed by DO tolerance limits. Highest feeding rates may thus coincide with low assimilation of nutrients from feed. Future studies may consider monitoring fish for avoidance behavior and responses to low DO conditions. Increased bacterial respiration and assimilation of nutrients, released by the feed, may cause the hypoxic zones to become more oxygen deprived, compounding the effects of hypoxia. These effects would be most observable in stagnant (non-mixed) ponds, because oxygen depletion would occur locally continuing to reduce already low oxygen concentrations. Aeration is an effective strategy to avoid occurrence of such hypoxic zones.

Aeration of ponds is commonly used by hatchery managers to combat low DO. Based on observations of this experiment aeration may be sufficient to mix ponds and increase DO near the kettle. Mixing also prevents growth of hypoxic zones. Unfortunately heavy aeration may have negative effects on pond environment. Accumulation of sediment in the center of the pond and erosion of pond bottoms are both reasons why managers should use aeration in moderation (Boyd 1998). Reduction in the amount of feed application to ponds may prevent the need for heavy aeration, thus avoiding its adverse effects on water quality.

Locally depleted oxygen near the kettle, coupled with the results of the BOD bottle experiment show that nutrients from feed may not be diffusing throughout the entire pond, but rather may be stimulating bacterial respiration locally near the kettle. This study did not address the extent of nutrient diffusion from feeding throughout the pond but this topic may be considered for future research.

Dissolved oxygen was measured in three separate ponds (a 3%, 1%, and 0% feeding rate pond), but there were no replicates within treatments. While this design achieves our goals by providing a very detailed snapshot of the spatial distribution of DO in the ponds at one point in time, it is insufficient to describe the temporal dynamics of changing DO conditions experienced during an entire culture season. Ideally, intensive sampling of pond DO could be done on a weekly basis for an entire culture season, giving researchers further understanding of the dynamics of DO within the pond system. Predictions about the differential effects of feeding regimens and management decisions could be more strongly supported using replicate ponds in future studies.

From a management stand-point, if fish are prevented from accessing feed they are likely to experience reduced growth and survival. Artificial feeding is implemented to promote fish growth, but indirect effects of feeding can lead to hypoxic zones which may prevent fishes' access to the feed. This paradoxical relationship between artificial feeding and fish growth causes inefficiencies of fish production. The questions which remain to be answered are: what amount of feed maximizes fish growth without limiting the oxygen availability around feeding areas, and what is the proper frequency of aeration which maximizes mixing, to reduce localized hypoxic zones, while also preventing erosion and accumulation of waterborne sediment?

Objective 2: The effects of artificial feed on nitrogen and phosphorus dynamics in fish ponds

Based on rapid release of nitrogen and phosphorus in the first hour of incubation in pond water, it is likely that nitrogen and phosphorus are released through a chemical dissimilation mechanism, rather than organic release. N:P of 10:1 is under the target weekly restoration rates in percid ponds (Jacob and Culver 2010), suggesting that artificial feed releases excessive phosphorus into the pond environment. Nitrogen may become the limiting nutrient in these ponds due to excessive release of phosphorus. Fish culture ponds, through high nutrient loading and nitrogen-limitation, have ideal conditions for growth of cyanobacteria, which gains competitive advantage over other phytoplankton in unmixed ponds (Culver 1991, and Levich 1996) due to their unique nitrogen fixation and buoyancy regulation (Paerl and Tucker 1995). Cyanobacteria are a poor food source for zooplankton and benthic invertebrates, which are key prey items for juvenile fish. By this mechanism, cyanobacteria effectively halt bottom-up productivity by preventing primary consumers from assimilating nutrients. Such disruptions in trophic dynamics may indirectly lead to reduced growth and survival of fish. Our study did not examine how nitrogen and phosphorus from feed was assimilated through the trophic food web, but future studies may consider stable isotope monitoring, or mass balance approaches to gain further understanding of this concept.

Sinking feed, such as the feed used in this experiment (Silver cup no. 3), may deliver nutrients to the benthos promoting growth of benthic filamentous algae. Filamentous algae such as *Rhizoclonium* sp., from a management stand-point, interfere with harvesting by forming floating mats on surface water when temperatures increase late in the culture season (Culver 1996). Entanglement of fish in mats during harvest can reduce the number of harvestable fish thus reducing fish yield. Such floating mats are subject to high amounts of sun radiation leading to large scale “die-offs,” resulting in accumulation of dead algae in pond sediments. Bacterial respiration consumes oxygen in the process of breaking down dead algae and leads to hypoxic conditions on the bottom of ponds. In this manner, excessive growth of cyanobacteria can reduce growth and survival of fish, leading to inefficiencies of aquaculture. Much of the nitrogen and phosphorus from feed not assimilated by fish may be delivered to, and accumulate in, the sediment (Dodds 2003), to be released in future culture seasons. Release of nutrients into future culture ponds can cause similar water quality issues such as growth of nuisance algae and reduction of DO availability. Aquaculture managers can avoid such conditions by reducing input of artificial feed and by mixing ponds more regularly.

Objective 3: Effects of $CuSO_4$ application on the consumption and distribution of DO

CuSO4 is often used by managers to control excessive growth of primary producers, and treat parasitic infections (*I. multifiliis*) in fish (Flemming and Trevors 1989, and Schlenk et al. 1998). In dark BOD bottles, reduced respiration, after $CuSO_4$ treatment, could be explained by reduced biomass of phytoplankton or bacteria. While $CuSO_4$ reduces consumption of oxygen in these closed systems, it also reduces primary productivity resulting in reduced DO availability for fish (Tucker and Boyd 1978) which is consistent with the results of this study. After treatment with $CuSO_4$, ponds showed not only a decrease in average DO concentrations, but also the absence of isolated zones of higher DO which may have previously acted as habitat refuges for fish. The entire areas monitored for DO in the 1% and 3% feeding rate ponds were uniformly hypoxic (Figure 3). Explanations for this drop in DO concentrations are best explained by a sharp decrease in overall primary productivity in the pond due to reduced phytoplankton biomass (Tucker and Boyd 1978). $CuSO_4$ does not reduce the demand for oxygen, but with low primary productivity it is expected to reduce the supply. Therefore, after addition of $CuSO_4$ one could expect to see a reduction in the already low DO, which is consistent with the results of our study. Based on these observations one could argue against the use of such a harsh chemical to treat the parasites, but this could be problematic to hatchery management because of the reduced growth rate associated with high energetic costs (parasite load) of fighting off the infectious *I. multifiliis*.

Management Suggestions

We recommend that fish aquaculture managers consider the negative effects of artificial feeding and $CuSO_4$ when designing feeding regimens for culture fish. We have shown that artificial feeding reduces DO concentrations locally in fish ponds, and increases DO consumption (in BOD bottle tests), creating hypoxic zones near the site of application. Excessive feeding may prevent fish from accessing feed, and low DO may reduce fish growth and survival (Andrews et al. 1973, and Boyd et al. 1978). Our study also showed that nitrogen and phosphorus are released from artificial feed in a 10:1 molar ratio which may promote growth of nuisance algae (Culver 1991, and Smith 1993) which can cause problems for managers at harvest and reduce growth and survival of fish. We showed that $CuSO_4$ reduces DO in fish ponds and other studies have documented other negative effects, such as storage in sediment that may carry over into other culture seasons (Han et al. 2001, and Tucker and Boyd 1978). For these reasons, we recommend designing feeding regimens which balance fish growth with suitable water quality, and avoiding excessive use of $CuSO_4$. Future studies may consider measuring spatial variation in DO temporally over an entire culture period, monitoring avoidance behavior of fish in ponds with hypoxic zones near the site of feeding, and using stable isotopes or mass balance approaches to more fully understand how nitrogen released from feed affects trophic dynamics. The negative effects of artificial feeding and $CuSO_4$ must be considered when designing strategies for fish culture management.

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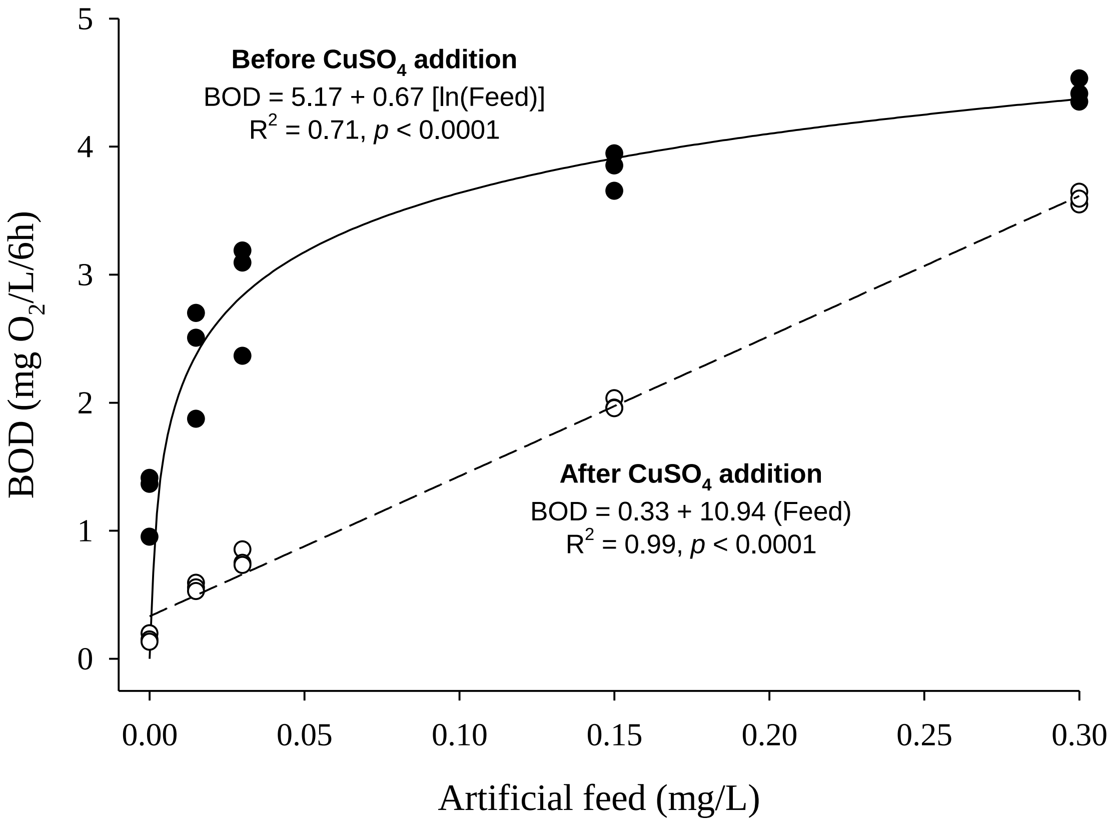
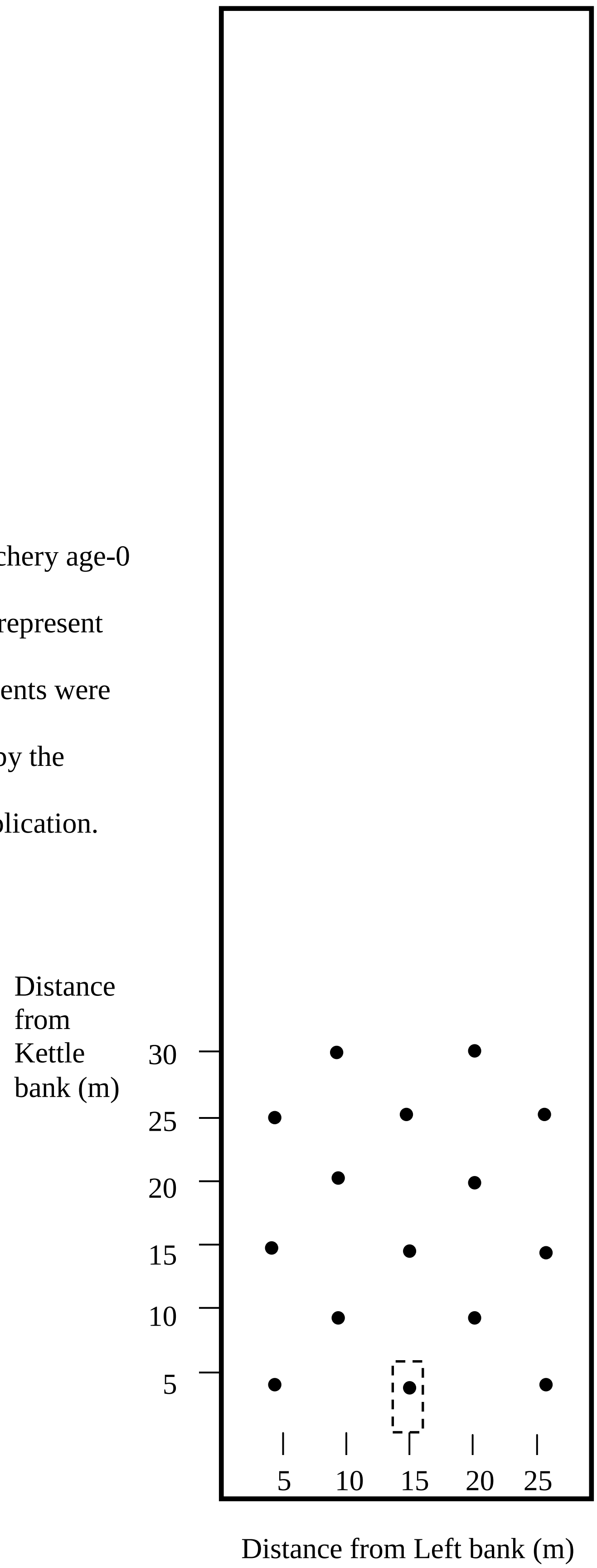


Figure 1: Comparison of the effects of artificial feed addition on BOD before and after copper sulfate addition to the 1% feeding rate pond. Mean BOD rates were comparatively greater before copper addition for each level of artificial feed tested (independent samples t-test, two-sided $p < 0.05$). Best fit lines are shown for before (solid) and after (dashed) copper sulfate treatment. A curvilinear relationship between feed addition and BOD was exhibited before, and a linear relationship after copper sulfate addition.

Figure 2: Scale line drawing of Hebron hatchery age-0 channel catfish nursery pond. The dark circles represent locations where DO and temperature measurements were taken within the pond. The kettle, represented by the dotted line, is also the location of daily feed application.



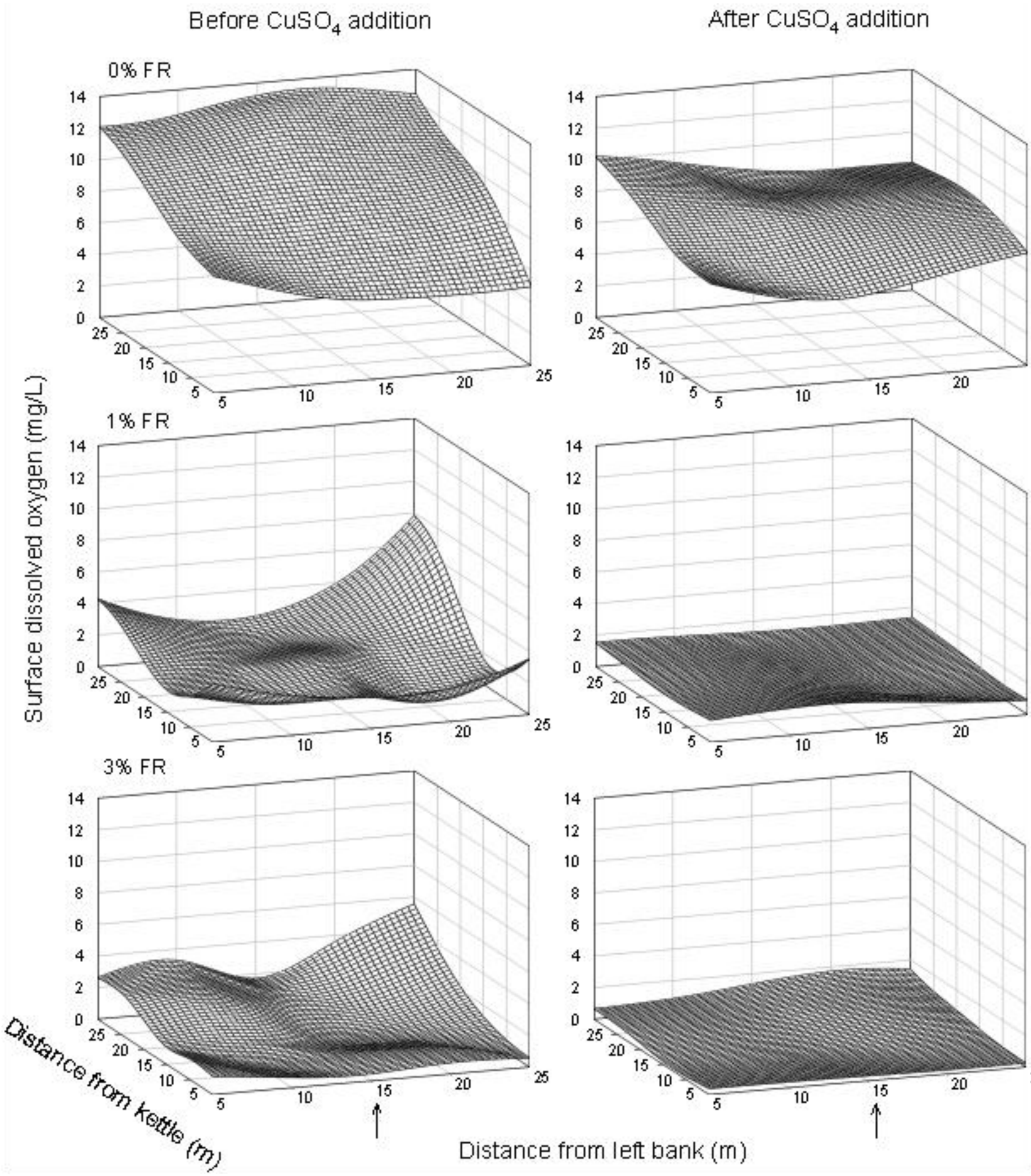


Figure 3: Mesh plots of DO spatial distribution as a function of feeding rate before and after copper sulfate application. Notice the lower DO concentrations throughout the 1% and 3% ponds as compared to the 0% pond, and the reduction in variability in the copper treated ponds. Arrows indicate the location of the kettle (location of feed

addition) where DO is often the lowest in fish ponds. Interpolation was performed by SigmaPlot (Systat Software, Inc., Chicago, Illinois).

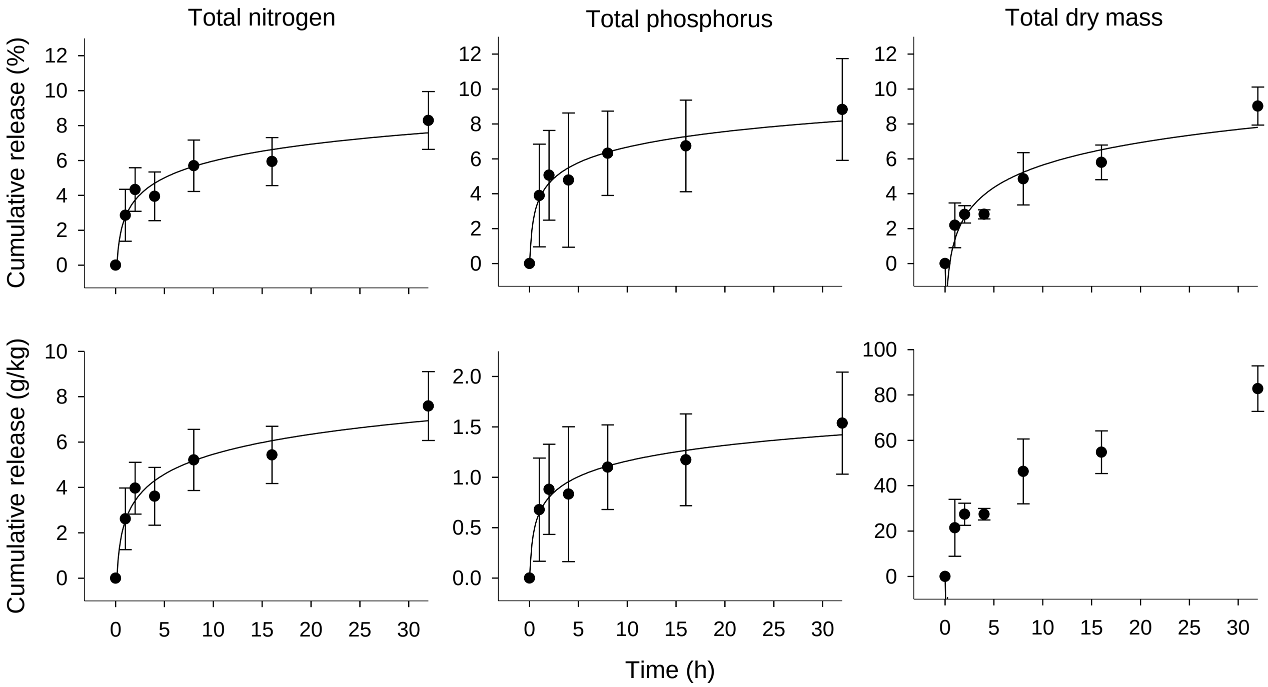


Figure 4: (Top) Comparison of cumulative percent of total N and P released from feed held in mesh bags over a 32 hour period in the 1% feeding rate pond at Hebron State Fish Hatchery, September 2010. (Water temperature was 20-22°C). Percentages are not significantly different between N, P or total dry mass release from feed. (Bottom) Comparison of masses per kg of feed of N, P and total mass released from feed over a 32 hour period in the 1% feeding rate pond at Hebron State Fish Hatchery, September 2010. Molar ratio of N:P released from feed remains constant through time at 10:1.

Table 1: Medians and ranges of DO concentrations, measured in mg L^{-1}, at the surface and bottom of fish ponds before and after copper sulfate additions for each feeding rate treatment. Medians were calculated from data points across a 30 m by 30 m area of the pond (Figure 2).

		Before Copper Sulfate		After Copper Sulfate	
Treatment		Surface	Bottom	Surface	Bottom
0%	Median	9.4	9.8	8.1	7.7
	Range	5.0 - 13.2	4.2 - 13.0	5.4 - 9.6	4.8 - 10.0
1%	Median	2.2	1.1	1.3	0.9
	Range	0.4 - 6.6	0.2 - 4.3	0.9 - 1.7	0.4 - 1.5
3%	Median	1.1	0.4	0.5	0.4
	Range	0.3 - 3.7	0.1 - 2.4	0.2 - 1.7	0.1 - 1.7